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(54) **GLUCOSE OXIDASE VARIANTS AND METHODS OF USING THE SAME**(71) Applicant: **Roche Diabetes Care, Inc.,**
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(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

- | | | |
|----------------|-----------------------|------------|
| 5,516,671 A * | 5/1996 Lawrence | A01N 63/00 |
| | | 435/69.1 |
| 6,376,210 B1 * | 4/2002 Yuan | C12Q 1/25 |
| | | 435/18 |
| 8,999,140 B2 * | 4/2015 Kojima | C12Q 1/006 |
| | | 204/403.01 |

FOREIGN PATENT DOCUMENTS

EP	354441 A2	2/1990
EP	2415863 A1	8/2012

OTHER PUBLICATIONS

- Altschul, Stephen et al., Basic Local Alignment Search Tool, *J. Mol. Biol.*, 1990, 403-410, 215.
Altschul, Stephen et al., Gapped Blast and PSI-Blast: a new generation of, *Nucleic Acids Research*, 1997, 3389-3402, 25, No. 17, Oxford University Press.
Bankar, Sandip et al., Glucose oxidase An overview, *Biotechnology Advances*, 2009, pp. 489-501, 27.
Bentlex, R. et al., The Mechanism of the Action of Notatin, *Biochem. J.*, 1949, 584-590, 45.
Berchmanns, Sheela et al., Layer-by-layer assembly of 1,4-diaminonaphthalimide, *Materials Chemistry and Physics*, 2002, pp. 390-396, 77, Central Electrochemical Research Institute, Karaikudi.
Bhatti, H.N. et al., Purification and thermodynamic characterization of glucose oxidase from a newly isolated strain of *Aspergillus niger*, *Canadian Journal of Microbiology*, 2006, 519-524, vol. 52.
Dennig, Alexander et al., OmniChange: The Sequence Independent Method for, *Plos One*, 2011, Issue 10, e26222, 6.
Frederick, Katherine R. et al., Glucose Oxidase from *Aspergillus niger*, *The Journal of Biological Chemistry*, 1990, 3793-3802, vol. 265, Issue of Mar. 5, 1990.
Gietz, Daniel et al., High-efficiency yeast transformation using the, *Nature Protocols*, 2007, pp. 31-34, 2, No. 1, Nature Publishing Group.
Hayashi, Sueko et al., Multiple Forms of Glucose Oxidase With Different Carbohydrate Compositions, *Biochimica et Biophysica Acta*, 1981, pp. 40-51, 657, Elsevier/North-Holland Biomedical Press.
Hecht, H.J. et al., Crystal Structure of Glucose Oxidase from *Aspergillus niger* Refined at 2.3 Angstrom Resolution, *J. Mol. Biol.*, 1993, pp. 153-172, 229, Academic Press Limited.
Hecht, H.J. et al., The 3D structure of glucose oxidase from *Aspergillus niger*. Implications for the use of GOD as a biosensor enzyme, *Biosensors & Bioelectronics*, 1993, pp. 197-203, 8, Elsevier Science Publishers Ltd.
Hoenes, Joachim et al., The Technology Behind Glucose Meters: Test Strips, Diabetes Technology & Therapeutics, 2008, pp. S-10-S-26, vol. 10, Supplement 1.
Horaguchi, Yohei et al., Turning glucose oxidase into essentially dehydrogenase, *Honolulu PRIME*, 2012, 2057, MA2012-02, Issue 18, The Electrochemical Society.
Lehle, Ludwig et al., Glycoprotein biosynthesis in *Saccharomyces cerevisiae*: ngl29, an N-glycosylation mutant allelic to och1 having a defect in the initiation of outer chain formation, *FEBS Letters*, 1995, pp. 41-45, 370, Federation of European Biochemical Societies.
Leskovac, V. et al., Glucose oxidase from *Aspergillus niger*: the mechanism of action, *The International Journal of Biochemistry & Cell Biology*, 2005, pp. 731-750, 37, Elsevier Ltd.

(Continued)

Primary Examiner — Hope Robinson**ABSTRACT**

Novel glucose oxidase (GOx) variants are disclosed that have the substitutions of T30V and I94V set forth in SEQ ID NO:1, and additionally at least one further amino acid substitution in the enzyme sequence in any of the positions S53; A137; A173; A332; F414 and V560. The GOx variants herein exhibit specificity for glucose and significantly reduced oxygen consumption rates and/or increased enzyme activity for electron mediators other than oxygen. Also provided are assay devices incorporating at least one of the GOx variants herein for improved blood glucose measurements.